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STUDIES ON THE POLLEN GRAINS OF RECENT CASTANEOIDEAE. II

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Abstract

During our transmission electron microscope investigations on recent *Castaneoideae* taxa, Ubish bodies, extratapetal membrane, and pellicula was observed outer the pollen grains. The exine of all the investigated species is tectate and perforated with channels. The infratectal layer is columellar and beneath the foot layer there is a granular endexine, in each case lamellar in the apertural area. In the apertural area, the ectexine is thinner than extragerminally. The contour of the cavern is the consequence of the refraction of light of the cavity between the ectexine and endexine. The general aspect of the ultrastructure of the ectexine and the endexine is identical with those of the earlier described extant pollen grain of *Castanea* type. Proved by the SEM data, the surface of the pollen grains of the *Castaneoideae* taxa is not psilate, but ornamented with short striae. This characteristic feature separates well from the brevaxonate *Amentiflorae* pollen grains, which are ornamented by characteristic coni.

Key words: Palynology, recent, Castaneoideae, TEM and SEM.

Introduction

In an earlier paper (KEDVES, 1982), the light microscope results of the *Castaneoideae* pollen grains are presented. As it was pointed out in this paper, the light microscope data should be completed by electron microscope results. This is the aim of this paper.

Previous TEM and SEM data were published by TAKEOKA (1965), MARTIN and DREW (1969), HESSE (1978), CREPET and DAGHLIAN (1980) and LIEUX (1980). TEM data of dispersed extant pollen grains were published by KEDVES and PÁRDUTZ (1973) and CREPET and DAGHLIAN (1980).

Materials and Methods

The material for our investigations was described in the previous paper. For TEM studies, acetolyzed and non-acetolyzed pollen grains were used, and were prepared with OsO_4. For SEM investigations non-acetolyzed pollen grains were mounted on polyvinylchlorid adhesive material then covered by gold-palladium. The pictures were taken in the EM Laboratory of the Institute of Biophysics, Biological Research Center of the Hungarian Academy of Science on a JEM-ASID scanning supplement of a JEOL-100B electron microscope.

Results

1. Transmission electron microscope results

1. *Castanea americana* RAF. (Plate I, Figs. 1, 2)

Interapertural exine. — The exine consists of ectexine and granular endexine. The ectexine is tectate, perforate, much thicker than the endexine. The three layers of the

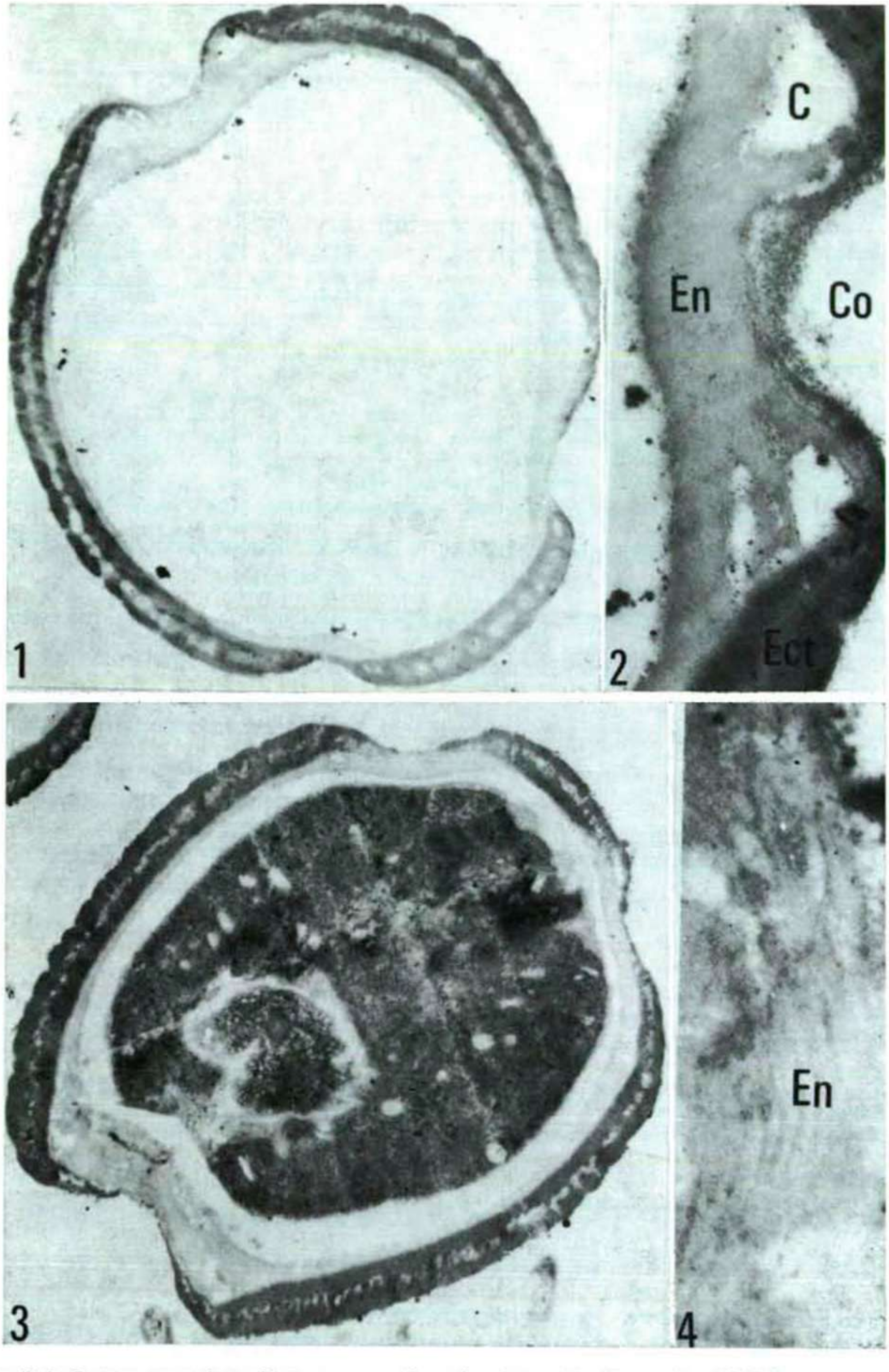

Plate I. 1. *Castanea americana* RAF., cross section of acetolyzed pollen grain, x10 000.
2. *Castanea americana* RAF., ultrastructure of the apertural area, x20 000.
3. *Castanea sativa* MILL., non-acetolyzed pollen grain, cross section, x10 000.
4. *Castanea sativa* MILL., lamellar endexine in the apertural area, x40 000.
C = cavern, Co = colpus, Ect = ectexine, En = endexine.

ectexine (tectum, infratectum and foot layer) are equal. The infratectal layer is columellar.

Apertural area. — Near the furrows, the ectexine is extremely thin, and the endexine is thick. In the colpal and the colporal area, the ectexine is granular (Plate I, Fig. 2, Co), and without threefold structure. The endexine becomes thick here, the ectexine protrudes out, and between the ect- and endexine there is a cavity (C).

2. *Castanea sativa* MILL. (Plate I, Figs. 3, 4, Plate II, Fig. 1)

Interapertural exine. — It consists of ectexine and endexine. The ectexine is tectate and channeled. The tectum and the foot layer are of equal thickness, the infratectum is a little thinner than the previously mentioned two layers. The fine structure of the infratectum is columellar. The endexine is granular, its thickness corresponds roughly to that of the infratectal layer.

Apertural area. — In the region of the furrows the ectexine becomes thinner. This thinning is uneven, the inner layers become thinner or disappear than the tectum. The endexine is much thicker than in the interapertural area. The cavern near the colpori forms as described previously, the ectexine becomes mostly a granular, not pluri-layered wall. In comparison to *C. americana* the lamellar ultrastructure of the endexine in the apertural area is worth mentioning (cf. NABLI, 1979).

3. *Castanopsis argyrophylla* KING (Plate II, Fig. 2)

Interapertural exine. — It consists of ectexine and granular endexine. The tectum in some places channeled. The infratectal layer is columellar, the three layers of the ectexine are equal.

Apertural area. — The ectexine near the furrows becomes extremely thin. The three ectexinal layers may not be recognized in this thin part. But the endexine is thick in this area. Near the colpori the ectexine is essentially identical with those of the colpi, but the thin more or less homogeneous part is a little longer. The cavern is essentially identical with the previously discussed ones but in this case the number of the cavities may be more than one. In the germinal area the ultrastructure of the endexine is granular.

4. *Castanopsis indica* DC. (Plate II, Fig. 3)

Interapertural exine. — It consists of ectexine and granular endexine. The ectexine is tectate, and based on our observations imperforate. Between the three ectexine layers the tectum is the thickest, the infratectal layer and the foot layer are equal. The infratectum is columellar.

Apertural area. — Near the furrows the ectexine become thinner, first of all the tectum, the endexine is only a little thicker than in the interapertural area. The apertural ectexine of the colpori is homogeneous, the endexine is granular, without lamellation. The cavern consists generally of one large and more smaller cavities.

5. *Castanopsis longispicata* HU (Plate II, Fig. 4, Plate III, Figs. 1—4)

At this species the ultrastructure of the elements which enclose the pollen grains was also suitable for investigations (Plate II, Fig. 4, Plate III, Fig. 1). Concerning the identification of the non exinous ultrastructural elements we are deeply indebted to Mr. PROF. DR. J. R. ROWLEY (Stockholms Universitet, Botaniska institutionen, Stockholm). We have observed numerous Ubish bodies, based on the literature data, its shape alters in the course of its growth. In our material we cannot observe these different forms, the bodies figured in this paper are probably the mature forms. As regards the origin of the Ubish bodies, see the papers of ROWLEY and ERDTMAN (1967) and ROWLEY and SKVARLA (1974). Based on the latter mentioned paper, the peculiar form of the Ubish bodies is determined by the glycocalyx of the plasma mem-

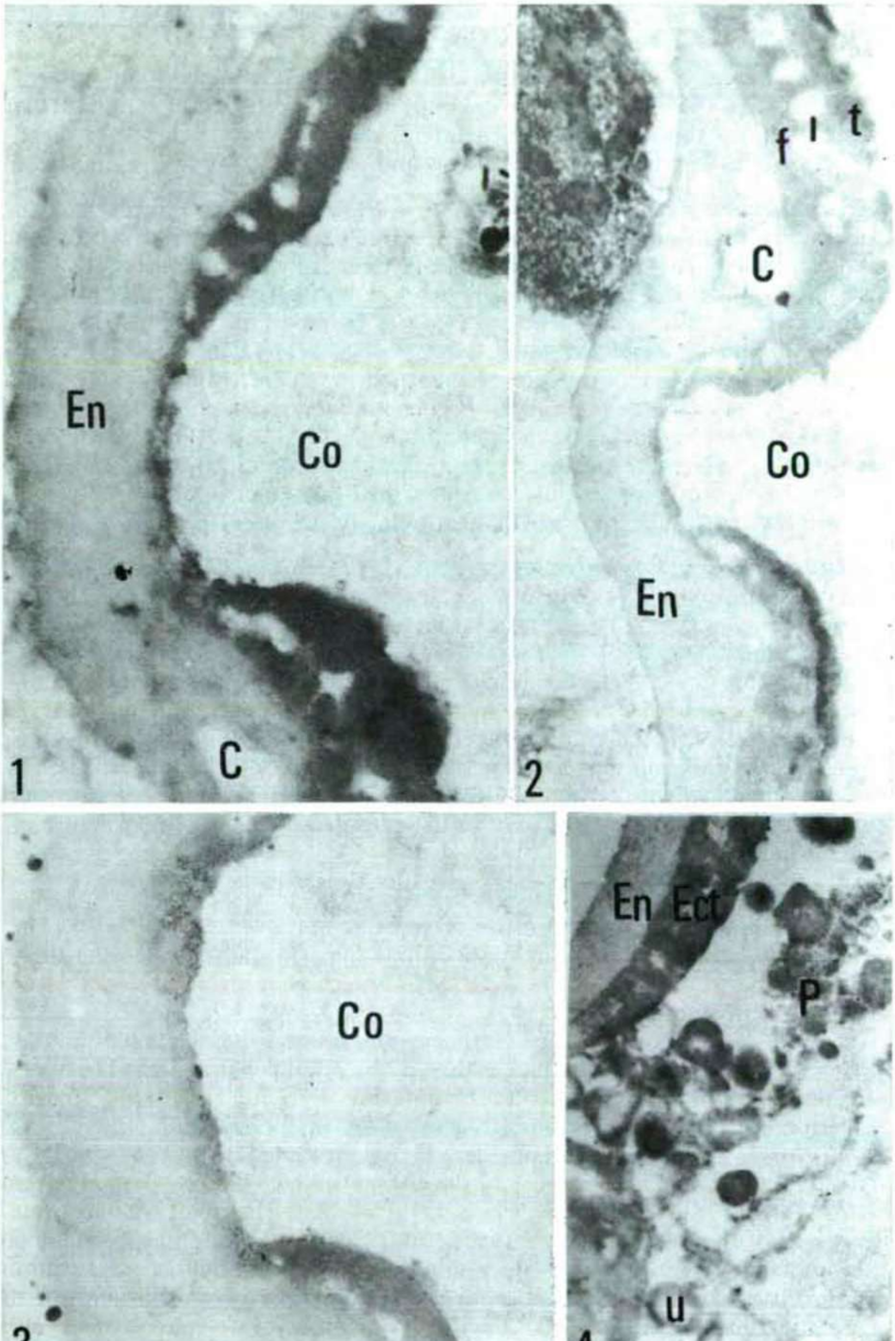

Plate II. 1. *Castanea sativa* MILL., ultrastructure of the apertural area, x40 000.
2. *Castanopsis argyrophylla* KING, ultrastructure of the apertural area, x20 000.
3. *Castanopsis indica* DC., ultrastructure of the apertural area, x40 000.
4. *Castanopsis longispicata* HU, ultrastructure of the exine and of the elements which enclose the pollen grains, x20 000.
C = cavern, Co = colpus, Ect = ectexine, t = tectum, i = infratectum, f = foot layer, En = endexine, P = pellicule U = Ubish bodies.

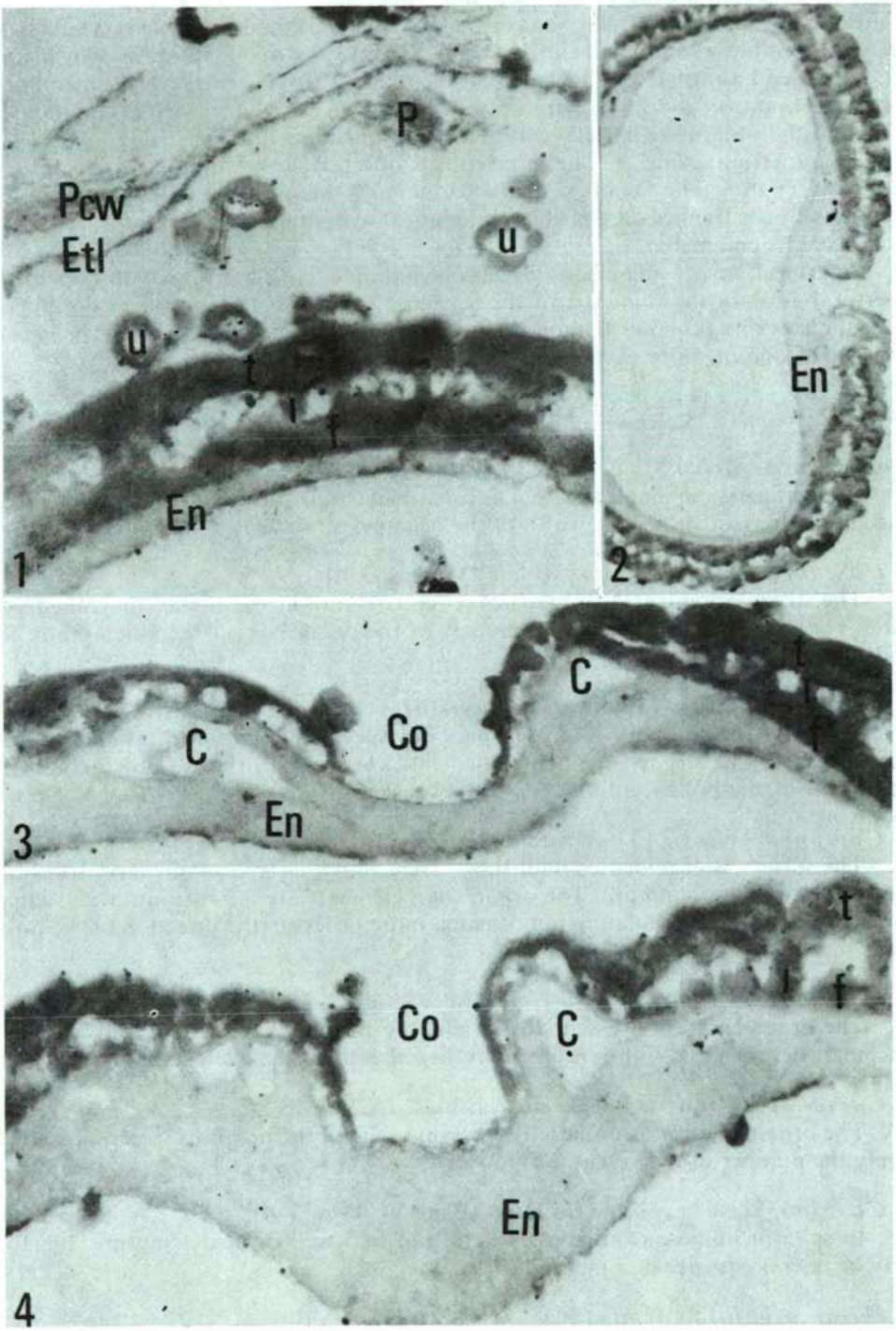

Plate III. 1. *Castanopsis longispicata* HU, ultrastructure of the exine and of the elements which enclose the pollen grains. x20000.
2. *Castanopsis longispicata* HU, longitudinal section of acetolyzed pollen grain, x10 000.
3, 4. *Castanopsis longispicata* HU, ultrastructure of the apertural area, x20 000.
C = cavern, Co = colpus, t = tectum, i = infratectum, f = foot layer, En = endexine, Etl = extra tapetal lamellation, P = pellicule, Pcw = parietal cell wall, U = Ubish bodies.

brane. In connection with the question of its function the following papers are worth mentioning: ECHLIN (1971), DUNBAR (1973), VASIL (1973) and MASCARENHAS (1975). Its taxonomic value was pointed out by UENO (1959). Moreover, residues of pellicules (cf. CERCEAU-LARRIVAL and ROLAND-HEYDACKER, 1976) = extratapetal membrane (HESLOP-HARRISON, 1969) = extratapetal lamellation (ROWLEY, a letter communication) and the wall of the parietal cell occured in our ultra-thin sections.

Interapertural exine. — The exine consits of ectexine and granular endexine, this latter is very thin. The thickness of the tectum and the foot layer is equal, the infratectum is a little thinner, its thickness is identical with that of the endexine. The infratectal layer is columellar.

Apertural area. — The ectexine becomes thinner, the endexine is thicker in the apertural area. In the thin part of the germinal exine first the foot layer disappears after the ectexine becomes homogeneous, and near the endopori divide. The cavern consists of one or more cavities.

2. Scanning electron microscope results

1. *Castanea sativa* MILL. (Plate IV, Figs. 1, 2)

The surface is very finely striate. The ornamental elements are diagonally oriented, and bifurcate frequently. The width of the ornamentation is 0.1—0.2 μm.

2. *Castanea americana* RAF. (Plate IV, Figs. 3, 4)

The striate ornamentation may be recognized only in the highly magnified pictures. The short striae are oriented obliquely of the polar axis of the pollen grain, the width is about 0.2 μm.

3. *Castanea pumila* MILL. var. *angustifolia* (Plate IV, Figs. 5, 6)

Similarly to the above discussed previous species, the ornamentation is not so characteristic. The striate ornamental elements are likevise oriented obliquely to the polar axis of the pollen grain.

4. *Castanea evansii* ELM. (Plate V, Figs. 1, 2)

The surface is ornamented with short striae, but this is in some places transitional to the rugulate sculpture. The ornamental elements are 0.2—0.3 μm wide, sometimes bifurcate, and undulating but oriented more or less in the direction of the polar axis.

5. *Castanopsis longispicata* HU (Plate V, Figs. 3, 4)

The ornamentation is essentially striate, the elements are 0.2—0.3 μm wide and irregular. Between this sculpture elements there are often small verrucae.

6. *Castanopsis indica* DC. (Plate VI, Figs. 1, 2)

The ornamentation is essentially the same as with the previous species, but relatively the number of the verrucae is lower.

7. *Castanopsis argyrophylla* KING (Plate VI, Figs. 3, 4)

In spite of numerous attempts we did not find well defined sculpture, but this may be in consequence of a methodical error.

8. *Pasania calathiformis* (SKAN.) H. et C. (Plate VII, Figs, 1, 2)

The surface is rugulate, foveolate, and only rarely striate, the width of the ornamental elements is 0.3—0.4 μm.

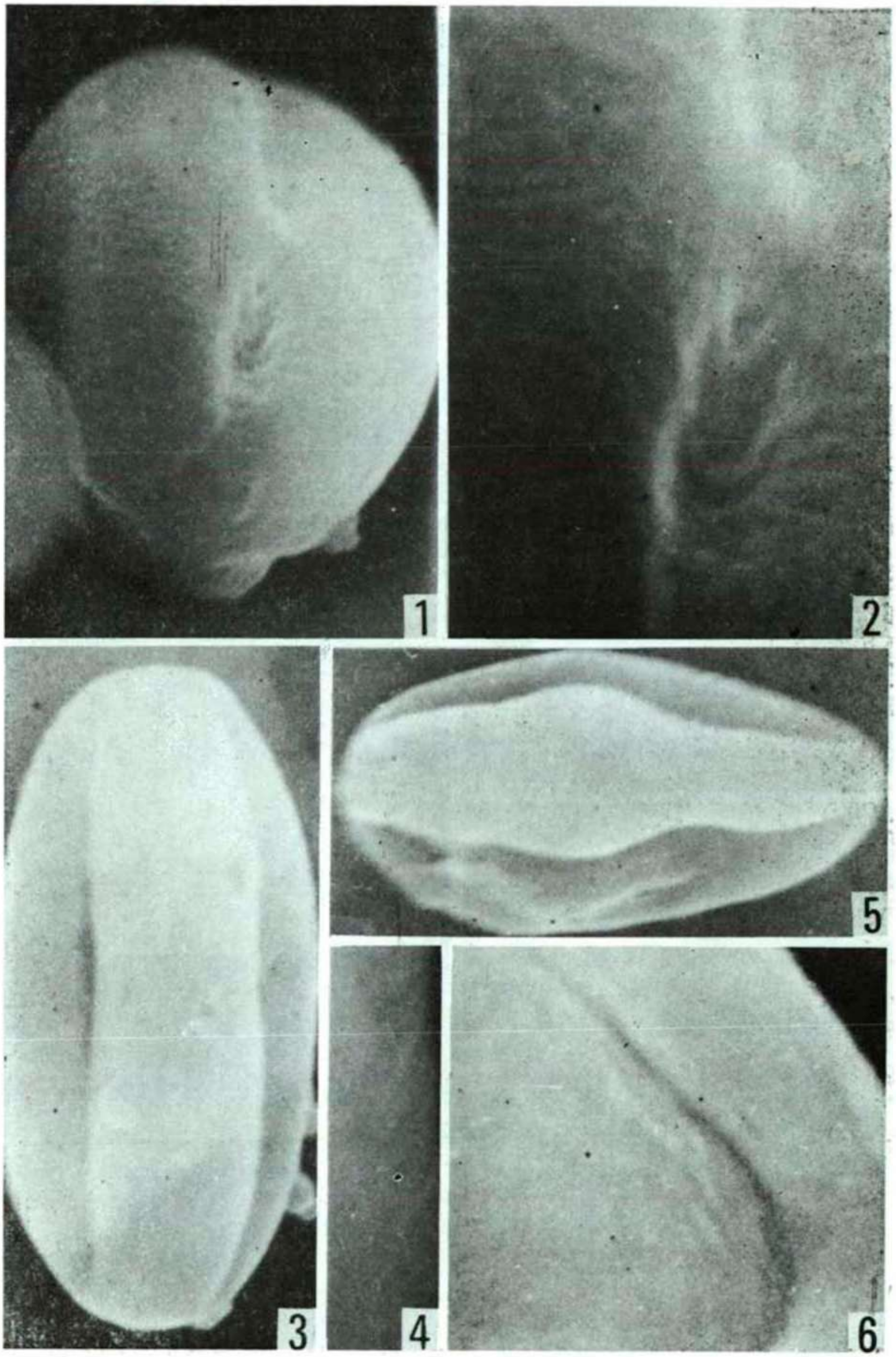

Plate IV. 1. *Castanea sativa* MILL., x5000.
2. *Castanea sativa* MILL., x10 000.
3. *Castanea americana* RAF., x5000.
4. *Castanea americana* RAF., x10 000.
5. *Castanea pumila* MILL. var. *angustifolia*, x5000.
6. *Castanea pumila* MILL. var. *angustifolia*, x10 000.

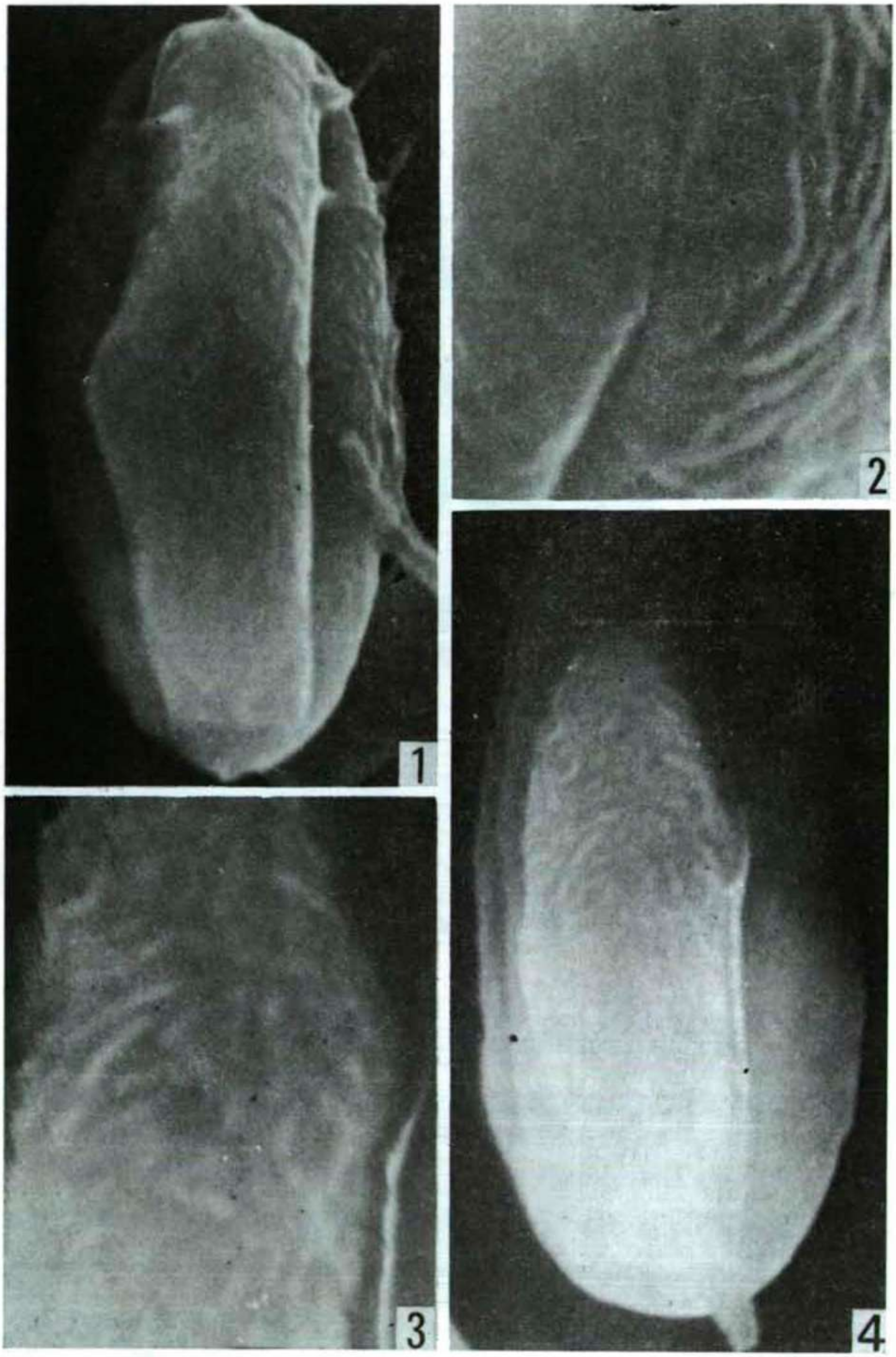

Plate V. 1. *Castanea evansii* ELM., x5000.
2. *Castanea evansii* ELM., x10 000.
3. *Castanopsis longispicata* HU, x10 000.
4. *Castanopsis longispicata* HU, x5000.

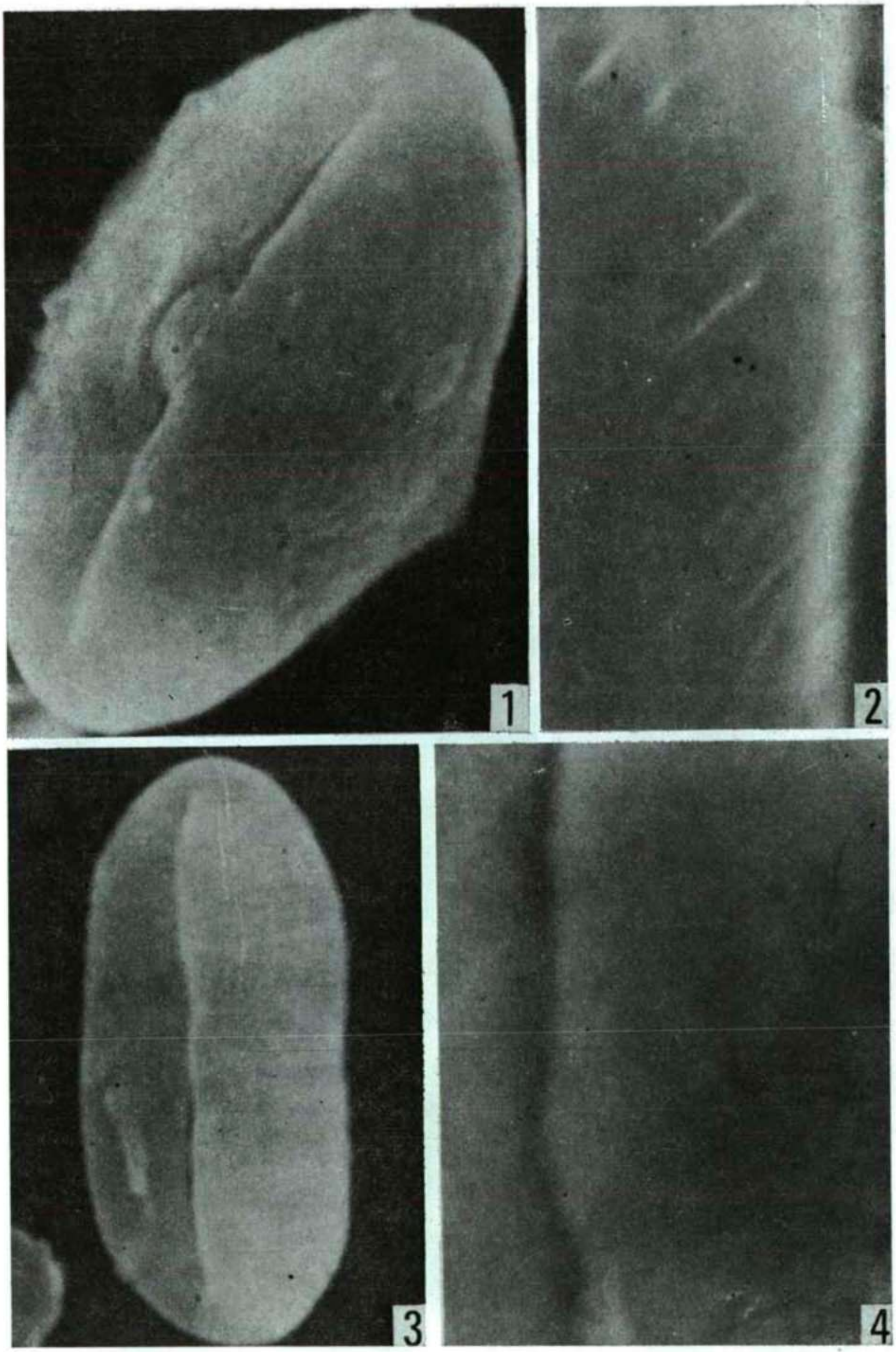

Plate VI. 1. *Castanopsis indica* DC., x5000.
2. *Castanopsis indica* DC., x10 000.
3. *Castanopsis argyrophylla* KING, x5000.
4. *Castanopsis argyrophylla* KING, x10 000.

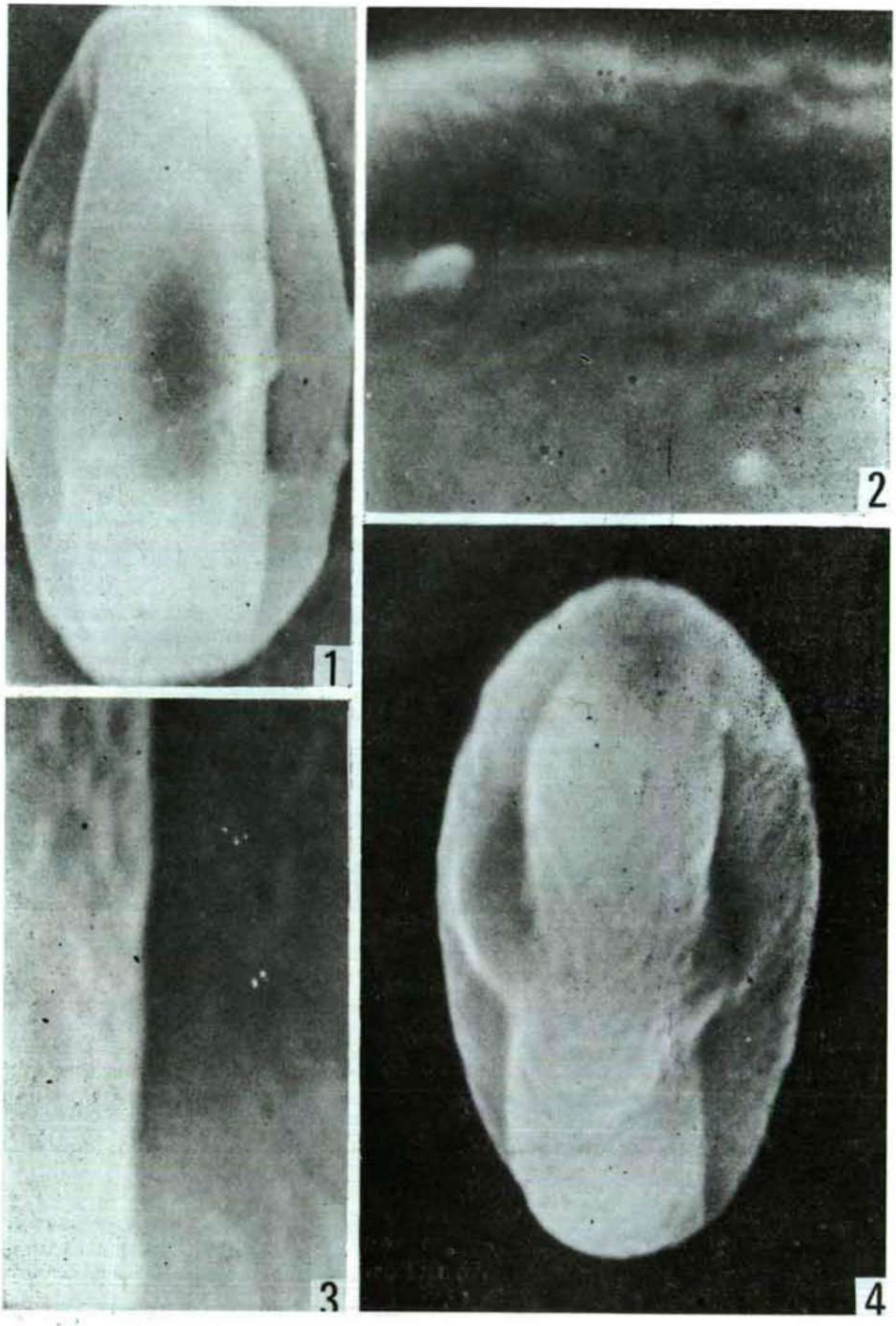

Plate VII. 1. *Pasania calathiformis* (SKAN.) H. ET C., x5000.
2. *Pasania calathiformis* (SKAN.) H. ET C., x10 000.
3. *Chrysolepis chrysophylla* (A. DC.) HJELMQVIST, x10 000.
4. *Chrysolepis chrysophylla* (A. DC.) HJELMQVIST, x5000.

9. *Chrysolepis chrysophylla* A. DC. (Plate VII, Figs. 3, 4)

The ornamentation is similar to the previous species, there is only one difference, the striate ornamentation is more frequent at this species.

Discussion

From pollen morphological point of view, the TEM structure of the caverns around the furrows is important, which is essentially in consequence of the refraction of light of the cavity (or cavities) between the ect- and endexine, or inside the endexine. This result is a partial ultrastructural interpretation of the classical lamellar ectexine stratification concept.

It is the submicroscopic striate surface, and the columellar infratectal layer which have taxonomic importance, and well separate the *Castaneoideae* pollen grains from the other *Amentiflorae* (cf. TAKEOKA 1965, DUPONT and DUPONT 1972, UENO 1975).

In respect to the evolution of the exine ultrastructure the granular endexine is worth mentioning, which is more developed, than the lamellar. But the occasional occur rence of the lamellae in the germinal area is interesting. For the importance of the lamellae the following papers are worth mentioning: CHANDA and ROWLEY (1967), ROWLEY and DUNBAR (1967), ROWLEY and SOUTHWORTH (1967), and M. VAN CAMPO and LUGARDON (1973). Endexine data about fossil *Castaneoideae* pollen grains are know from the Eocene, it may be concluded that there is no essential difference between recent and fossil endexine fine structure.

As a general conclusion it may be emphasized, that the submicroscopic characteristic features of the exine of the brevaxonate and longaxonate *Amentiflorae* pollen grains alternate with the symmetry of the polar axis. The occurrence of the endexine, and the ultrastructure of the infratectum of the different groups have different evolutionary or taxonomic significance.

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